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13. ABSTRACT ***(Maximum 200 Words)***

The objective of this project is the development of a PET radiopharmaceutical for measuring multidrug resistance (mdr) in breast cancer. Multidrug resistance is resistance of a lesion to a specific class of drugs that includes many of the chemotherapeutics that are most effective against breast cancer. Single-photon myocardial perfusion agents such as ^{99m}Tc-MIBI are substrates for Pgp, the protein implicated in mdr, and are now being studied for evaluation of mdr. A PET mdr tracer would provide significant advantages over ^{99m}Tc-MIBI, and the half-life of ^{64}Cu (12.7 h) is better matched to the apparent biological half-life of the mdr process in breast cancer (~240 min.) than are other PET radionuclides (e.g., ^{11}C, $T_{1/2}$= 11 min). We are carrying out *in vivo* and *in vitro* studies of lipophilic cationic ^{64}Cu-based PET radiopharmaceuticals as potential PET mdr radiopharmaceuticals using murine (MAT-B) and human (MCF-7) breast cancer models. *In vitro* studies of prototype ^{64}Cu complexes reveal a pattern of uptake similar to that observed for ^{99m}Tc-MIBI. Studies are currently underway to determine the optimal chemical properties of this agent. The development of a radiopharmaceutical for the measurement of the mdr status of breast cancer lesions will facilitate optimization of treatment protocols, monitoring of the development of acquired resistance, and real-time evaluation of mdr modulators.

14. SUBJECT TERMS
Breast Cancer, PET, ^{64}Cu, Multidrug Resistance

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INTRODUCTION

The objective of this project is the development of a PET (Positron Emission Tomography) radiopharmaceutical for the quantitative functional evaluation of multidrug resistance (mdr) in breast cancer. Multidrug resistance (mdr) is defined as intrinsic or acquired resistance to a specific class of chemotherapeutic drugs that includes many of the most effective chemotherapeutic agents against breast cancer. Multidrug resistance is characterized by overexpression of the *MDR1* gene and increased concentration of its product, P-glycoprotein (Pgp), a 170 kD transmembrane glycoprotein, which acts as an efflux pump to reduce the intracellular concentration of these drugs to non-toxic levels. Lipophilic cationic complexes such as ^{99m}Tc-MIBI are substrates for Pgp and are now being studied for clinical evaluation of mdr. We are currently carrying out *in vivo* and *in vitro* studies of lipophilic cationic ^{64}Cu-based PET radiopharmaceuticals as possible PET mdr radiopharmaceuticals. A PET mdr tracer will provide significant advantages over ^{99m}Tc-MIBI (e.g., straightforward attenuation corrections, higher spatial resolution, greater sensitivity, and the ability to perform quantitative studies), and the half-life of ^{64}Cu (12.7 h) is better matched to the apparent biological half-life of the mdr process (~240 min.) than are other PET radionuclides such as ^{11}C ($T_{1/2}$= 11 min). Furthermore, ^{11}C-based radiopharmaceuticals are only available at a limited number of institutions and, because of the short half-life of ^{11}C, can not be shipped to other institutions. These radiopharmaceuticals will provide real-time information about the mdr status of breast cancer lesions that may allow optimization of treatment protocols, monitoring of the development of acquired resistance, and evaluation of the effectiveness of drugs developed to modulate mdr.

BODY

The research accomplishments are discussed in terms of each Task outlined in the Revised Statement of Work (6/1/99) relevant to the first 24 months of the Project (8/1/99-7/31/01). Note that, as described in detail under Task 1, the technician who was originally recruited to work on this project left after approximately one year, and the vacant position was not filled until June 2001. Accordingly, although this report covers the second fiscal year of the project, it actually describes progress on the project during the six-month period from August 1, 2000 to December 1, 2000 and June 1, 2001 through July 31, 2001 when a technician was available to address the project's Tasks.

Task 1: Recruitment and training of research technician, Months 1-3

Ms. Erica A. Guice, the technician who was recruited in October 1999, resigned in December, 2000. Prior to and after her departure an extensive effort was made to recruit a new technician to fill this position but no acceptable candidates were identified until spring, 2001. The absence of acceptable candidates during this period was presumably due to the extremely low unemployment rate in the Boston area at that time and the paucity of recent science graduates looking for technical positions. In mid-spring 2001 approximately 50 applications were reviewed and interviews were conducted with six finalists. Following these interviews, Mr. Robert Borgesi, who received his B.S. degree in Biology from Boston College in May 2001, was hired to fill this position effective June 4, 2001.

During the first month of Mr. Borgesi's appointment he underwent required new employee training sessions including Lab Safety Training, Radiation Safety Training, Animal Use Orientation (including Small Animal Use) at both Children's Hospital and Harvard Medical School. During this time learned the techniques required to establish and maintain parental and drug resistant cell lines and the methods used to measure uptake of the new radiotracers in these cells.

Because of departure of Ms. Guice and unavailability of suitable candidates to immediately fill her position, there was a time lag of approximately six months in this project, the time between Ms. Guice's departure and the recruitment of Mr. Borgesi. Since the arrival of Mr. Borgesi, however, we have made rapid progress in the establishment and validation of the breast cancer cell lines.

Task 1 has been completed.

Task 2: Establish and validate *in vitro* assay for multidrug resistant breast cancer, Months 3-12

a. Establish breast cancer cell lines
b. Establish mdr breast cancer cell lines
c. Validate parental and mdr breast cancer cell lines with ^{99m}Tc-MIBI
d. Validate parental and mdr breast cancer cell lines with prototype ^{64}Cu PreH and cyclops complexes

Two breast cancer cell lines have been established, the rat MAT-B cell line and the human MCF-7 cell line. The MAT-B cell line is an ascites tumor derived from the rat 13762 solid mammary adenocarcinoma. This cell line has the advantage that is native to the Fischer rat and is, therefore, expected to show more physiologically relevant perfusion in *in vivo* studies. The MCF-7 is a human mammary adenocarcinoma cell line. This cell line is more relevant to the problem of human breast cancer, but *in vivo* studies must be carried out as xenografts in nude mice with the associated differences between the two species. This cell line was obtained from Robert Gillies at the University of Arizona and the Arizona Cancer Center and is currently being maintained in our laboratory.

The multidrug-resistant MAT-B cell line, MAT-B/r was established in Dr. Jones' laboratory from the parental cell line and is now available as needed. The multidrug-resistant MCF-7 cell line was, as with the parental, obtained from Dr. Gillies and the Arizona Cancer Center and is now being maintained in our laboratory. Since the recruitment of Mr. Borgesi, we have had no problems maintaining either of these cell lines.

Both the MAT-B and MCF-7 parental and drug resistant cell lines were validated *in vitro* with ^{99m}Tc-MIBI. These studies confirmed both the lower uptake of ^{99m}Tc-MIBI by the drug-resistant cells and the ability of Cyclosporin A to counteract drug resistance in these models.

Preliminary *in vitro* studies were carried out with the MAT-B and MCF-7 (resistant and parental) cell lines and the prototype ^{64}Cu PreH complex ($[^{64}Cu(Me_2MAKPreH)]^+$). The results of a typical study with the parental and resistant MCF-7 cell lines are shown graphically in the Figure 1 (next page).

In summary, the parental and resistant MCF-7 cells were equilibrated with $[^{64}Cu(Me_2MAKPreH)]^+$ at 37° C, samples were removed at selected time intervals, and the uptake of ^{64}Cu by the cells was measured. The experiment was also carried out using media containing 1 μM Cyclosporin A, a known mdr reversal agent. As can be seen from Figure 1, the uptake of the ^{64}Cu complex by the resistant cell line (-▼-) is low relative to that of the parental cell line (-●-). In the presence of Cyclosporin A, however, there is no difference between the uptake of the ^{64}Cu complex by the parental (-○-) and resistant (-▽-) cell lines. This result supports the hypothesis that the uptake of these cells is lower in drug-resistant breast cancer cells than in non-resistant cells, which in turn suggests that these complexes may be useful for PET evaluation of multidrug resistance in breast cancer. Additional studies are planned to evaluate the mechanism of uptake as well as to determine the optimal chemical properties for the copper complex.

At the present time, no studies are being carried out with the ^{64}Cu-"cyclops" derivatives. These complexes offer no apparent advantage over the PreH derivatives and are more difficult to prepare and purify at the no-carrier-added level.

Task 2 has been completed.

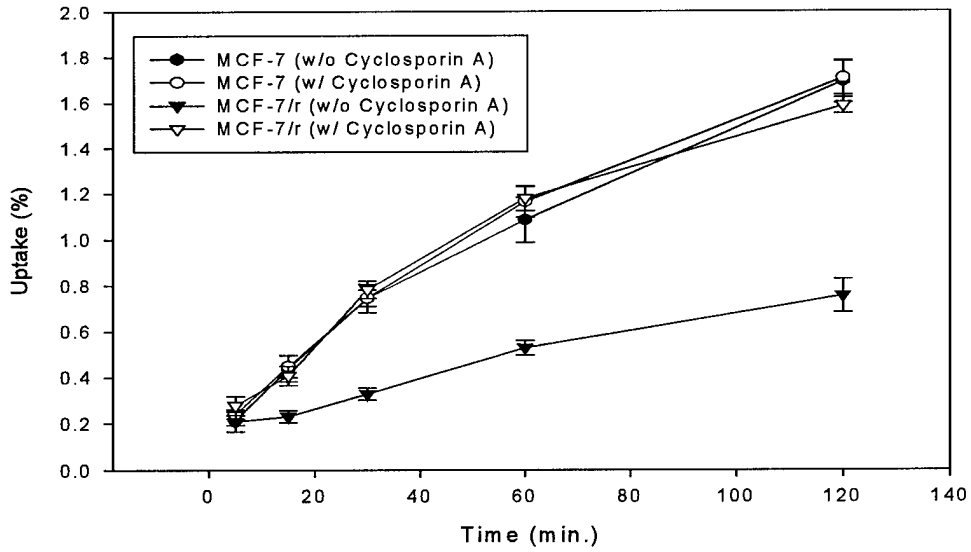

Figure 1. Uptake of $^{64}Cu(Me_2MAKPreH)$ by MCF-7 Cells

Task 3: Establish and validate *in vivo* assay for multidrug resistant breast cancer, Months 3-12

a. Establish breast cancer cell lines in animal model
b. Establish mdr breast cancer cell lines in animal model
c. Validate parental and mdr breast cancer cell lines with ^{99m}Tc-MIBI
d. Validate parental and mdr breast cancer cell lines with prototype ^{64}Cu PreH and cyclops complexes

The parental and resistant breast cancer cell lines have been established, and it is now possible to begin the *in vivo* studies. An amendment was recently submitted to the existing animal protocol to allow us to carry out studies using the parental and drug-resistant MAT-B cell lines simultaneously in the same animal. It is anticipated that this amendment will be approved within the next several weeks at which time the animal studies will be initiated. At that time we will first carry out a study in this model with ^{99m}Tc-MIBI to validate the model. As soon as the *in vivo* model is validated, studies will be carried out with the prototype ^{64}Cu PreH complexes. It is expected that the first *in vivo* studies with this model will be completed within the next six to eight weeks.

Parts a and b of this task have been completed, and parts c and d will be completed within the next six to eight weeks.

Task 4: Evaluate the effect of chemical properties (lipophilicity, ligand geometry, ligand substituents) on the biological properties of a copper-based PET radiopharmaceutical for the functional assessment of multidrug resistance in breast cancer, Months 13-24

a. *In vitro* studies of the ^{64}Cu-labeled complexes using breast cancer model
b. *In vivo* studies of the ^{64}Cu-labeled complexes using breast cancer model

We have prepared and characterized more than 25 new ^{64}Cu-labeled monocationic complexes (Fig. 2).

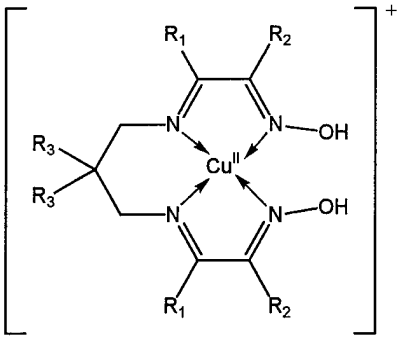

Figure 2. Cu(II) Diiminedioxime Complex

The lipophilicity (log *P*) of these complexes varies from approximately –2 to +2, as a function of the substituents R_1, R_2, and R_3, a difference of four orders of magnitude. The substituents at R_1 and R_2 vary from methyl through *n*-heptyl; R_3 is either H or methyl. We have also prepared a small number of complexes where R_1 and R_2 are aryl groups. The *in vitro* and *in vivo* testing of these complexes had been delayed because of the previously reported problems with the breast cancer cell lines. These problems have been resolved, and we can now test four to six of these complexes per month. This testing rate is predicted on the bimonthly shipment of ^{64}Cu from Washington University and the testing of two to three complexes per shipment. At this rate we will complete the testing of all of the currently available complexes in four to six months.

Only the most promising of these complexes will be tested *in vivo*. These studies will use two separate animal models. The primary model will be the MAT-B (rat) model described above. This model will be used for standard biodistribution studies in which the test compound is injected into tumor-bearing animals that are then sacrificed at predetermined times to measure the uptake of the complexes by the tumor. These studies will be initiated as soon as the amended animal protocol (amendment submitted 8/20/01) is approved by the Children's Hospital IACUC.

Additional studies will be carried out using nude mice bearing MCF-7 tumors as indicated by the results of the studies with the MAT-B model.

Part a of this Task (*in vitro* studies) is now being carried out with completion anticipated in four to six months. Part b of this Task (*in vivo* studies) will begin in September and continue as promising compounds are developed. Taking into account the six-month period during which no technician was available, this Task is on schedule to be completed approximately on schedule.

Task 5: Evaluate differences between biological properties of the ^{64}Cu PET radiopharmaceuticals in breast and non-breast mdr tumor models, Months 13-24

In vitro data have been collected for more than 20 ^{64}Cu complexes using the MES-SA parental and resistant cell lines. These results will be compared to the results for the MAT-B parental and resistant cell lines as the MAT-B data is acquired over the next four to six months. This Task will be completed at approximately 26-28 months, approximately on schedule.

Task 6: Integrate results of Task 4 and Task 5 into the development of a ^{64}Cu-based PET radiopharmaceutical for the evaluation of mdr in breast cancer, Months 13-36.

This Task will continue throughout the project.

KEY RESEARCH ACCOMPLISHMENTS

- Recruited and trained replacement research technician
- Established tissue culture facility in Children's Hospital Nuclear Medicine Laboratory
- Established parental and drug-resistant MAT-B (rat) breast cancer cell lines
- Established parental and drug-resistant MCF-7 (human) breast cancer cell lines
- *In vitro* validation of parental and drug-resistant MAT-B (rat) breast cancer cell lines with ^{99m}Tc-MIBI
- *In vitro* validation of parental and drug-resistant MCF-7 (human) breast cancer cell lines with ^{99m}Tc-MIBI
- *In vitro* validation of parental and drug-resistant MAT-B (rat) breast cancer cell lines with prototype ^{64}Cu-Me_2MAKPreH complex
- *In vitro* validation of parental and drug-resistant MCF-7 (human) breast cancer cell lines with prototype ^{64}Cu-Me_2MAKPreH complex

REPORTABLE OUTCOMES

Abstracts and presentations (Copies of the abstracts are appended to this report)

1. Packard AB, Kiani S, Guice E. "Synthesis and characterization of carrier-free ^{64}Cu diiminedioxime complexes: Potential PET radiopharmaceuticals for evaluating multidrug resistance." PacifiChem 2000, Honolulu, HI, December, 2000, #MEDI-66.
2. Kiani S, Packard AB. "Radiosynthesis and *in vitro* study of ^{64}Cu-labeled diiminodioxime complexes as putative PET imaging agents for evaluation of multidrug resistance (mdr).", 12th International Radiopharmacology Symposium, Interlaken Switzerland, June, 2001
3. Kiani S, Packard AB. "The development and in vitro characterization of lipophilic cationic copper(II) complexes as potential PET radiopharmaceuticals for the functional evaluation of multidrug resistance.", NERM 2001, Durham, NH, June, 2001

Cell lines

The MES-SA and MES-SA/Dx5 (human uterine sarcoma) cell lines are established and being used for method validation.

The parental and multidrug-resistant MAT-B (rat mammary adenocarcinoma) cell lines are established and validated.

The parental and multidrug-resistant MCF-7 (human mammary adenocarcinoma) cell lines are established and validated.

CONCLUSIONS

After some delays in year-01, we have now successfully established two pairs of cell lines, MAT-B and MCF-7, for use in the *in vivo* and *in vitro* evaluation of the target ^{64}Cu complexes. These cell lines were validated using ^{99m}Tc-MIBI and the prototype ^{64}Cu complex, $[Cu(Me_2MAKPreH)]^+$. The copper complex showed a similar pattern of uptake to that of ^{99m}Tc-MIBI complex. Although these results are preliminary, they demonstrate that the pattern of uptake of this complex parallels the drug resistant status of the tumor cells, which supports our hypothesis that these complexes may prove useful as PET radiopharmaceuticals for the evaluation of mdr in breast cancer. Additional *in vitro* studies are carried out biweekly. These results are being added to a database that will be used to develop structure/biodistribution relationships that will guide the development of new copper complexes. The *in vivo* evaluation of the first ^{64}Cu complexes will be carried out within the next six to eight weeks. These data will also be added to the database as they are accumulated.

The project is now on schedule to complete the evaluation of the ^{64}Cu complexes as originally scheduled.

REFERENCES

N/A

APPENDICES

Abstracts

1. Packard AB, Kiani S, Guice E. "Synthesis and characterization of carrier-free ^{64}Cu diiminedioxime complexes: Potential PET radiopharmaceuticals for evaluating multidrug resistance." PacifiChem 2000, Honolulu, HI, December, 2000, #MEDI-66.
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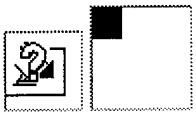

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Abstract

MEDI 66

Synthesis and characterization of carrier-free ^{64}Cu diiminedioxime complexes: Potential PET radiopharmaceuticals for evaluating multidrug resistance.

A. Packard*, S. Kiani, E. Guice, E. Guice, *Harvard Medical School, Nuclear Medicine, 300 Longwood Avenue, Boston, MA, 02115, USA, Fax: 617-232-0517

The aim of this work is to develop ^{64}Cu-based PET radiopharmaceuticals for the functional evaluation of multidrug resistance in breast cancer and other malignancies. We previously identified the lipophilic cationic copper(II) complexes of the diiminedioxime ligands as promising candidates for this application using low specific-activity ^{64}Cu (2 mCi/mg) and carried out preliminary biological studies that confirm the potential utility of these complexes for this application. We have now developed a rapid synthesis (<5 min.) for use with high specific-activity (HSA) ^{64}Cu (>10^5 mCi/mg) that produces these complexes in 90% yield and 90% radiochemical purity and characterized the products by HPLC and ITLC using the "cold" complexes as standards. In vitro stability was tested by equilibration of the ^{64}Cu-complex with 2.5% BSA/PBS using a Sephadex G-50 column, which revealed no evidence of plasma binding or transchelation of ^{64}Cu to albumin. Additional in vivo and in vitro studies are currently underway.

ABSTRACT FORM: 12th International Symposium of Radiopharmacology, June 12-15, 2001 - Interlaken (Switzerland)

RADIOSYNTHESIS AND IN VITRO STUDY OF ^{64}CU-LABELED DIIMINODIOXIME COMPLEXES AS PUTATIVE PET IMAGING AGENTS FOR EVALUATION OF MULTIDRUG RESISTANCE (MDR)

S. Kiani, A. Packard, Children's Hospital, Harvard Medical School, Boston, MA, USA

The aim of this study is to develop a copper-based PET compound for the evaluation of multidrug resistance (mdr) in cancer. For this purpose, we investigated the effect of variations in the alkyl substituents on the cellular accumulation of a series of ^{64}Cu complexes of pseudomacrocyclic diiminedioxime ligands. The uptake of the ^{64}Cu complexes was measured in vitro in the MES-SA uterine sarcoma cell line and its multidrug-resistant DX5 derivative. The effect of the mdr reversal agent Cyclosporin A on the uptake was also measured. The stability was tested by equilibration of the ^{64}Cu-complex with human serum/PBS, which revealed no evidence of plasma binding or transchelation of ^{64}Cu to serum proteins. A systematic increase in cell uptake was observed as the length of the alkyl substituent (and thus the lipophilicity) increased from methyl (log P = -2) through pentyl (log P = 2). There was a corresponding increase in the difference between the uptake by resistant and non-resistant cells. These results suggest that ^{64}Cu diiminedioxime complexes are promising agents for PET evaluation of MDR.

TITLE IN CAPITAL LETTERS
Authors' names, preceded by initials in lower cases; underline presenting authors' name. Institution name in lower cases.

Text should contain: a) aim of the study; b) methods; c) summary of the results; d) discussion and conclusion. Do not include references. Type the text including any tables in single line spacing. Take care not to type outside the blueline. Use standard abbreviations. Place unusual abbreviations in parentheses after the full word the first time it appears. Clearly identify all radio-pharmaceuticals used; standard abbreviations may be used (MDP, HMPAO, MIBI, etc.). For good reproduction the typing should be sharp and regular, using courier type 12. Please do not use smaller type because the abstract will be reduced to 70% during the reproduction process.

The author(s) will be informed of the decision of the Scientific Committee at the address for correspondence before **April 15, 2001**. Abstracts which have been accepted will be published in *The Quarterly Journal of Nuclear Medicine*. Abstracts which duplicate or slightly modify previous work or submitted to more than one topic will be automatically rejected by the Scientific Committee. Abstracts which are not suitable for reproduction will not be published in the proceedings.

Deadline for receipt of Abstracts: January 31, 2001

The original abstract typed on this form and 10 anonymous copies must be sent to the following address:

Professeur Denis Guilloteau
Laboratoire de Biophysique Médicale et Pharmaceutique
INSERM U316
UFR de Pharmacie
31 Avenue Monge
37200 Tours - FRANCE

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Abstract title Radiosynthesis and in vitro study of ^{64}Cu-labeled diiminodioxime complexes as putative PET imaging agents for MDR
Name of presenting author Alan B. Packard, Ph.D.
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164. THERMODYNAMICS OF COPPER(II) BINDING TO BOVINE SERUM ALBUMIN.

Dean Wilcox and Yi Zhang, Department of Chemistry, 6128 Burke Laboratory, Dartmouth College, Hanover, NH 03755.

New insight about metal metabolism and metal toxicity will require a better understanding of the interaction of metal ions with proteins, and we have begun studies to quantify the thermodynamics of these interactions. Isothermal titration calorimetry (ITC) has been used to measure metal binding to the protein bovine serum albumin (BSA) and to a three-residue peptide corresponding to the N-terminal BSA sequence, which is the primary Cu(II) binding site. We find that the Cu(II) affinity of the BSA N-terminal site is modulated by the properties of cysteine-34. When this residue is oxidized or chemically derivatized the Cu(II) affinity decreases by nearly an order of magnitude, and this decreased affinity is predominantly due to entropic factors (greater loss of entropy upon Cu(II) binding). We also find that a second Cu(II) binds to Cys-34 of BSA at pH 9.2, and the coordination of the Cu(II) bound at this site has been characterized by UV-vis, CD and EPR spectroscopy. Finally, the temperature dependence of the enthalpy change (ΔH) upon Cu(II) binding to BSA and to the N-terminal tri-peptide has been measured to quantify the contribution of solvent interactions to the thermodynamics of metal binding to this protein.

165. SYNTHESIS AND IN VITRO STUDY OF ^{64}Cu-LABELED DIIMINODIOXIME COMPLEXES FOR EVALUATION OF MULTIDRUG RESISTANCE (MDR).

S. Kiani and A. Packard, Children's Hospital, Harvard Medical School, Division of Nuclear Medicine, 300 Longwood Avenue, Boston, MA 02115.

The development of multidrug resistance (MDR) is a major problem in chemotherapy. The objective of this project is the development of a lipophilic cationic copper-based PET compound for evaluation of MDR in cancer. As part of the optimization of this agent, we are investigating the effect of the variations in alkyl substituents on the uptake of ^{64}Cu complexes of diiminidioxime ligands by wild-type and drug resistant tumor cells. We have developed a rapid synthesis (<5 min.) for use with high specific-activity ^{64}Cu that produces these complexes in >90% yield and >90% radiochemical purity. The stability of these complexes was tested by equilibration of the ^{64}Cu-complex with human serum/PBS. Accumulation of the ^{64}Cu complexes in the human uterine carcinoma cell line MES-SA and its doxorubicin resistant DX5 derivative was measured. Addition of MDR reversal agent, cyclosporin A did not substantially affect uptake of the ^{64}Cu complexes in MES-SA cells but increased the accumulation in resistant cells to approximately the same value as the parental cells. This difference increases as the lipophilicity of the complexes increases. These results suggest that ^{64}Cu-diiminodioxime complexes are promising agents for PET evaluation of MDR.